



The influence of tau, amyloid, alpha-synuclein, TDP-43, and vascular pathology in clinically normal elderly individuals



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ABSTRACT

Many individuals live to older ages without clinical impairment. It is unknown whether brain pathologies in these individuals are associated with subtle clinical deficits. We analyzed the brains of 161 clinically normal (Clinical Dementia Rating score = 0) older individuals enrolled in the Mayo Clinic Patient Registry or Study of Aging. We assessed for the presence and burden of beta-amyloid, tau, alpha-synuclein, TDP-43, and vascular pathology. We investigated whether pathologies were associated with antemortem cognitive and motor function, depression, MRI volumetric measures, or the apolipoprotein E (*APOE*) ε4 allele. Eighty-six percent had at least 1 pathology, and 63% had mixed pathologies. Tau and vascular pathology were associated with poorer memory scores. Tau was also associated with poorer general cognition scores and smaller amygdala, hippocampi, and entorhinal cortex volumes. Beta-amyloid neuritic plaque burden was associated with greater depression scores. The presence of a greater number of pathologies was associated with *APOE* ε4 carrier status and with poorer memory performance. Some dementia-related pathologies are associated with poorer performance in clinical measures and brain atrophy in the unimpaired elderly.

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1. Introduction

A proportion of the older adult population performs within a defined normal range on clinical measures of cognition, motor function, and depression. Such individuals are referred to, or considered as, normally aged individuals. Many such individuals have died while remaining normal and have undergone neuropathological examination of their brains. Interestingly, pathological brain examinations of these individuals have demonstrated findings ranging from no/minimal pathology to multiple-mixed pathologies (Bennett et al., 2006; Knopman et al., 2003; Matthews et al., 2009; Rahimi and Kovacs, 2014; SantaCruz et al., 2011; Suemoto et al., 2017). Many of the pathologies identified in these

normally aged individuals can also be found in individuals with progressive neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), among others.

There are 4 main proteins that are associated with neurodegenerative disease. These include beta-amyloid (Aβ), tau, alpha-synuclein, and the TAR DNA binding protein of 43 kDa (TDP-43). Aβ and tau are considered the 2 cardinal proteins that characterize AD, although alpha-synuclein and TDP-43 are commonly observed (Dickson, 2001; Josephs et al., 2014b); alpha-synuclein-associated pathologies characterize the Lewy body spectrum diseases, including PD (Dickson, 2001). Tau and TDP-43 can be associated with frontotemporal lobar degeneration (Mackenzie et al., 2010) and chronic traumatic encephalopathy (McKee et al., 2015), and TDP-43 is also associated with ALS (Neumann et al., 2006). Furthermore, at the time of death, it is common to find evidence of vascular pathology in the brains of older individuals (Fernando and

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Ince, 2004); however, the association between the presence, and burden, of these 5 pathologies ($A\beta$, tau, alpha-synuclein, TDP-43, and vascular pathology) and performance on clinical measures of cognitive function, motor function, or depression in individuals deemed clinically normal (CN) has not been extensively examined. It is also unclear whether a greater number of pathologies are associated with lower performance on clinical measures or with the apolipoprotein E (*APOE*) $\epsilon 4$ allele. Many of the neurodegenerative proteins and vascular pathology are associated with brain atrophy within populations of clinically impaired individuals, but whether they influence brain structure in normal aged individuals is another unknown. Given these gaps in knowledge, we designed a clinico-imaging-pathological study to assess the influence of the neurodegenerative brain proteins and vascular disease in a cohort of CN older subjects who had a postmortem brain examination.

2. Methods

2.1. Subject selection

We identified 161 subjects in our pathological database who had been recruited and prospectively followed up in the Mayo Clinic Alzheimer's Disease Patient Registry or the Mayo Clinic Study of Aging (MCSA), who underwent a brain autopsy between 1999 and 2015, were CN, and had a Clinical Dementia Rating Scale (Hughes et al., 1982) sum of boxes (CDR-SB) score of 0 at the last assessment before the patient died. For each participant, determination of CN status was based on consensus agreement between the study coordinator, examining physician, and neuropsychologist who evaluated the participant, taking into account education, prior occupation, visual or hearing deficits, and reviewing all other

participant clinical information. The Alzheimer's Disease Patient Registry was a community-based study of cognitive aging. The MCSA is a prospective population-based study of cognitive aging in Olmsted County, Minnesota (Roberts et al., 2008), and it has been shown that in the MCSA, autopsied participants were very similar to participants in the MCSA who have died without autopsy on a broad array of demographic and clinical features (Graff-Radford et al., 2018). All subjects had also undergone *APOE* genotyping. Demographic features of all 161 subjects are shown in Table 1. This study was approved by the Mayo Clinic Institutional Review Board. Before death, all subjects or their proxies had provided written consent for brain autopsy examination.

2.2. Pathological analysis

All 161 subjects had undergone pathological examination according to the recommendations of the Consortium to Establish a Registry for Alzheimer's disease (CERAD) (Mirra et al., 1991). The left hemibrain was fixed, and paraffin block sections were stained with hematoxylin and eosin and modified Bielschowsky silver stain. Immunohistochemistry was performed using antibodies for $A\beta$ (6F/3D; 1:10; Novocastra Vector Labs, Burlingame, CA, USA), phospho-tau (AT8; 1:1000; Endogen, Woburn, MA, USA), alpha-synuclein (LB509; 1:200; Zymed, San Francisco, CA, USA), and TDP-43 (polyclonal antibody [MC2085] that recognizes a peptide sequence in the 25-kDa C-terminal fragment). For TDP-43, we screened the amygdala and hippocampus to assess for the presence of neuronal cytoplasmic inclusions, dystrophic neurites, neuronal intranuclear inclusions, and perivascular inclusions. For each of the 161 cases, we determined whether the pathological lesions were negative (absent or scant) or positive (present and more than

Table 1
Subject demographics, neuropsychological scores, and imaging volumes

	All (n = 161)	Subjects with imaging (n = 102)
Demographics		
Female	86 (53%)	55 (54%)
Age at clinical examination, y	87 [81, 90] (62, 100)	88 [81, 91] (73, 100)
Age at MRI, y		87 [81, 90] (73, 99)
Age at death, y	89 [84, 93] (62, 103)	91 [84, 94] (74, 103)
Postmortem interval, h	15 [9, 20] (2, 59)	16 [10, 23] (2, 59)
Clinical examination to death, y	1.5 [0.68, 3.37] (0.04, 9.4)	1.6 [0.69, 3.31] (0.04, 9.4)
MRI to death, y		3.0 [1.3, 5.5] (0.1, 11.7)
Education, y	14 [12, 16] (6, 20)	14 [12, 16] (8, 20)
<i>APOE</i> $\epsilon 4$ carrier	37 (23%)	21 (21%)
Clinical scores		
Mini-Mental State Examination score/30	28 [27, 29] (23, 30)	28 [27, 29] (23, 30)
WMS Logical Memory Delayed Recall/50	17 [12, 24] (1, 35)	18 [13, 24] (1, 35)
Boston Naming/60	55 [51, 57] (24, 60)	55 [51, 57] (25, 60)
Controlled Oral Word Association Test	36 [28, 45] (9, 74)	36 [28, 45] (13, 74)
WAIS-Block Design/51	20 [15, 25] (0, 51)	20 [14, 25] (2, 51)
AVLT Delayed Recall/15	7 [4, 9] (0, 15)	6 [4, 9] (0, 15)
Trails A/180	47 [36, 62] (22, 125)	49 [38, 62] (22, 125)
Trails B/300	124 [92, 163] (56, 300)	130 [96, 170] (60, 300)
Total UPDRS/44	1 [0, 3] (0, 17)	1 [0, 3] (0, 17)
BDI-II grand total/63	6 [2, 9] (0, 19)	6 [2, 9] (0, 15)
Gray matter volume (cm³)		
TIV (l)		1.50 [1.40, 1.60] (1.08, 1.87)
Amygdala		1.98 [1.79, 2.17] (1.51, 2.75)
Hippocampus		7.06 [6.51, 7.52] (5.18, 8.62)
Entorhinal cortex		2.86 [2.46, 3.31] (1.61, 4.47)
Lateral frontal		55.1 [50.6, 60.1] (18.5, 80.8)
Lateral parietal		34.2 [30.8, 37.8] (12.6, 51.9)
Lateral temporal		65.0 [59.9, 70.6] (36.5, 88.7)
Medial frontal		27.4 [25.3, 29.9] (10.8, 38.4)
Medial parietal		18.5 [16.7, 21.1] (9.0, 28.7)

Data shown are median [quartiles] (range) or n (%).

Key: *APOE*, apolipoprotein E; AVLT, Auditory Verbal Learning Test; BDI-II, Beck Depression Inventory-II; WMS, Wechsler Memory Scale; TIV, total intracranial volume; UPDRS, Unified Parkinson's Disease Rating Scale; WAIS, Wechsler Adult Intelligent Scale.

scant), and we also assigned a stage for each pathological lesion. A β was considered positive if there was any evidence of A β senile plaques (neuritic, cored, or diffuse) in the frontal, temporal, parietal, or occipital cortex. Alpha-synuclein was considered positive if Lewy bodies were identified in any brainstem or cortical region. TDP-43 was considered positive if there were any TDP-43 immunoreactive inclusions in the amygdala or hippocampus. Tau was considered positive if there were neurofibrillary tangles (NFTs) present beyond the transentorhinal cortex (\geq stage II) (Braak and Braak, 1991). We chose to treat tau in this manner as there were only 4 cases without any NFTs. For vascular lesions, we reviewed the pathological records at the time of autopsy examination for the presence of microinfarcts and the presence of lacunar (<1 cm)/larger infarcts (\geq 1 cm). If any type of infarct was present, the case was considered positive for vascular pathology. We did not include cerebral amyloid angiopathy in our vascular pathology variable as this is a different type of vascular disease that is also highly correlated with the presence of A β and would have confounded our analyses. A subject was considered as having none, one, or multiple pathologies if they had none, one, or more than one of the pathologies as defined previously. In addition to a designation of present/absent or high/low, we also assessed all 5 pathological lesions based on the distribution/stage as an indirect measure of burden (Table 2). Therefore, each case was assigned a Braak NFT stage (Braak and Braak, 1991) and a CERAD neuritic A β stage (Mirra et al., 1991) using modified Bielschowsky silver stain, and a TDP-43 stage (Josephs et al., 2016) using TDP-43 (polyclonal antibody MC2085 that recognizes a peptide sequence in the 25-kDa C-terminal fragment). For Lewy body disease, we used the following modified Braak and Braak staging scheme for Lewy bodies (Braak et al., 2003): stage 0 = no Lewy bodies, stage 1 = Lewy bodies restricted to brainstem, stage 2 = Lewy bodies extending to limbic cortex, and stage 3 = Lewy bodies extending to neocortex. Four

stage 0 cases had amygdala-only Lewy bodies. For vascular stage, we used the following staging scheme: stage 0 = no vascular infarcts, stage 1 = microinfarcts or lacunar/large infarcts, and stage 2 = both microinfarcts and lacunar/larger infarcts present.

2.3. MRI analyses

Of the 161 subjects, 102 had completed a volumetric head MRI scan during life. Demographic features of all 102 subjects with MRI scans during life are shown in Table 1. We performed atlas-based parcellation using the automated anatomical labeling atlas to measure volumes of the amygdala, hippocampus, entorhinal cortex, lateral frontal lobe (superior frontal, middle frontal, inferior frontal operculum, inferior frontal triangularis), lateral parietal lobe (superior parietal, inferior parietal, supramarginal gyrus, angular gyrus), lateral temporal lobe (superior temporal, middle temporal, inferior temporal), medial frontal lobe (supplemental motor area, frontal superior medial, anterior cingulum), and medial parietal (precuneus, posterior cingulum) lobe at the last MRI scan before death.

2.4. Clinical and neuropsychological assessments

We analyzed the following clinical and neuropsychological variables for this study: Mini-Mental State Examination (Folstein et al., 1975) to assess general cognitive function, the Wechsler Memory Scale-Revised Logical Memory II delayed recall (Wechsler, 1987) and Auditory Verbal Learning Test (AVLT) (Rey, 1964) to assess verbal episodic memory, the Boston Naming Test (Kaplan et al., 1983) to assess confrontation naming, the Controlled Oral Word Association Test (Sumerall et al., 1997) to assess language, the Wechsler Adult Intelligence Scale-R Block Design (Wechsler, 1987) to assess visuospatial abilities, Trail Making Test Parts A and B (Reitan, 1958) to assess cognitive speed and executive function, a modified version of the Unified Parkinson's Disease Rating Scale (UPDRS, 2003) to assess for the presence and severity of Parkinsonism, and the Beck Depression Inventory-II (BDI-II) (Beck et al., 1961) to assess for depression.

2.5. Statistical analyses

Demographic variables were compared between those positive and negative for A β , tau, alpha-synuclein, TDP-43, and vascular disease. Groupwise comparisons of demographics were performed using Fisher's exact or Wilcoxon rank-sum tests, where appropriate.

Associations between pathological lesion and clinical outcomes were assessed using linear regression models, which incorporated all 5 pathologies as predictors, adjusting for age at clinical examination and years between examination and death. For MRI brain volumes, linear regression models were adjusted for age at MRI scan, duration from MRI scan to death, and log-transformed total intracranial volume (log-TIV). Two sets of linear regression models were performed, the first treating the pathological measures as binary variables (i.e., presence/absence) and the second as ordinal variables (i.e., pathological stage). We also compared clinical scores and MRI volumes between subjects with only microinfarcts and subjects with only lacunar/large infarcts using linear regression (subjects with both were excluded).

We assessed the differences in demographics, clinical test scores, and brain volumes between those without any pathology and those with at least one type of pathology. Associations with clinical test scores were assessed using linear regression adjusted for age at clinical examination and years from clinical examination to death. Associations with brain volumes were assessed using linear regression adjusted for age at MRI, years from MRI to death,

Table 2
Pathology distribution

	All (n = 161)	Subjects with imaging (n = 102)
β -Amyloid stage		
0	69 (43%)	44 (43%)
1	50 (31%)	30 (29%)
2	29 (18%)	19 (19%)
3	13 (8%)	9 (9%)
Tau stage		
0	4 (3%)	1 (1%)
1	20 (12%)	11 (11%)
2	53 (33%)	36 (35%)
3	41 (26%)	27 (27%)
4	25 (16%)	14 (14%)
5	16 (10%)	11 (11%)
6	2 (1%)	2 (2%)
α -Synuclein stage		
0	139 (87%)	88 (87%)
1	9 (6%)	7 (7%)
2	8 (5%)	4 (4%)
3	4 (3%)	2 (2%)
TDP-43 stage		
0	126 (81%)	76 (75%)
1	14 (9%)	13 (13%)
2	6 (4%)	4 (4%)
3	2 (1%)	2 (2%)
4	0 (0%)	0 (0%)
5	7 (5%)	7 (7%)
6	0 (0%)	0 (0%)
Vascular stage		
0	83 (52%)	49 (49%)
1	20 (13%)	16 (16%)
2	42 (26%)	26 (26%)
3	15 (9%)	10 (10%)

and log-TIV. Logistic regression adjusting for age at death was performed to evaluate the odds of at least one type of pathology for *APOE* ϵ 4 carriers versus noncarriers. Trend tests were also performed to evaluate the association between number of pathologies present or high (0, 1, 2, 3, 4, 5) and clinical test scores or brain volumes. Associations with clinical test scores were adjusted for age at clinical examination and years from clinical examination to death. Associations with brain volumes were adjusted for age at MRI, years from MRI to death, and log-TIV.

All statistical analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), version 3.4.4. Results were considered statistically significant at $p < 0.05$.

3. Results

In our cohort, approximately half (53%) of the subjects were female and 37 (23%) were *APOE* ϵ 4 carriers (Table 1). The most common pathology was A β , which was positive in 105 subjects (65%), followed by tau which was positive in 84 subjects (52%). The other pathologies were present in less than 50% of the subjects including alpha-synuclein ($n = 22$, 14%), TDP-43 ($n = 35$, 22%), and vascular pathology ($n = 77$, 48%). Age at death was greater in positive versus negative subjects for tau (median [IQR] = 91 [87, 94] vs. 88 [82, 91], $p < 0.001$), alpha-synuclein (93 [88, 95] vs. 89 [83, 92], $p < 0.01$), and TDP-43 (91 [89, 95] vs. 88 [83, 92], $p < 0.001$). A β (+) subjects were more likely to be *APOE* ϵ 4 carriers than A β (-) subjects (30% vs. 9%, $p < 0.01$), as were tau(+) subjects (32% vs. 13%, $p < 0.01$). The staging of all 5 pathologies is shown in Table 2. The most common neuritic A β stage was 0 with the majority of subjects ($n = 119$; 74%) having no-scant neuritic plaques. For Braak NFT stage, 143 subjects (89%) were \leq stage IV; only 2 subjects (1%) were stage VI. For alpha-synuclein and TDP-43, the majority of subjects (>80%) were stage 0. For vascular disease, 59 subjects (37%) had only microinfarcts, whereas 23 (15%) had only lacunar/larger infarcts; 8 subjects (5%) had both microinfarcts and lacunar/larger infarcts.

One-hundred thirty eight (86%) of the 161 subjects had at least one pathology, and 102 (63%) had multiple pathologies; some as many as 4 different pathologies (Fig. 1). The frequencies of the different combinations of pathologies are shown in Table 3. When assessing the co-occurrence of 2 pathologies, the most common pathology combination was A β and tau (40%), followed by A β and vascular (28%) and then tau and vascular (20%). Alpha-synuclein and TDP-43 rarely occurred together (3%). When assessing the co-occurrence of 3 pathologies, the most common combination was A β , tau, and vascular (16%).

In the linear regression models investigating each type of pathology adjusting for the other pathologies, we found that tau, both presence and burden, was independently associated with poorer Mini-Mental State Examination, Wechsler Memory Scale delayed recall, and AVLT delayed recall scores and smaller amygdala and entorhinal cortex volumes (Figs. 2–5). Tau burden (i.e., NFT Braak stage) was also associated with smaller hippocampal volumes (Fig. 5). We observed an association between higher A β CERAD stage and higher BDI-II scores (Fig. 4). We also found that the presence and burden of vascular pathology was associated with poorer AVLT scores (Figs. 2 and 4). None of the clinical and MRI outcome measures differed between subjects with microinfarcts compared with subjects with lacunar/large infarcts.

The linear regression models also showed some associations that could be considered counterintuitive. These counterintuitive results were almost exclusively observed when pathologies were treated as burden, rather than presence/absence. That is, we observed greater burden of pathology to be associated with better performance on clinical measures or larger MRI volumes. For *alpha-synuclein*, we observed a higher lesion burden to be associated with better

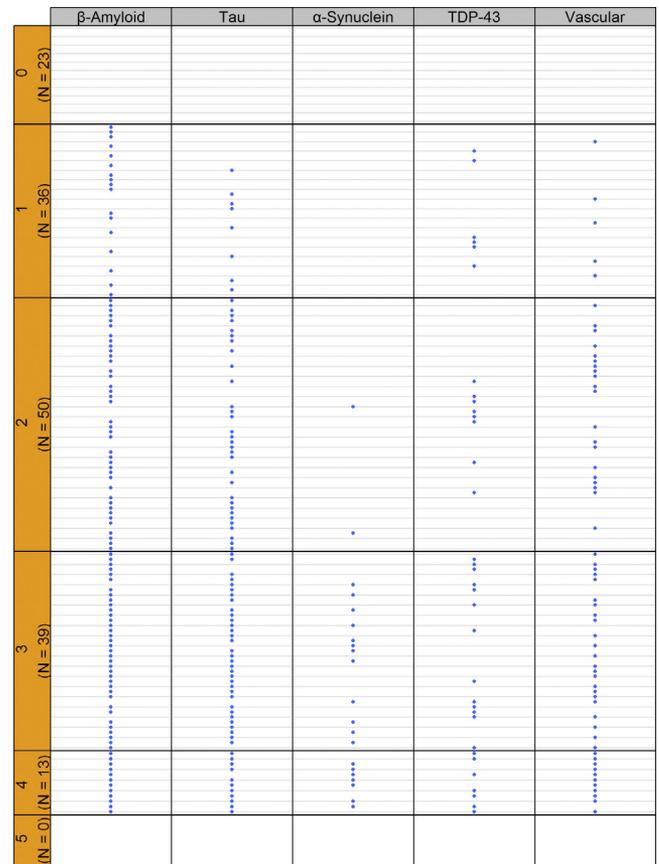


Fig. 1. Plot showing the different types of pathologies for each subject grouped by the number of pathologies.

performance on the Wechsler Adult Intelligent Scale block design (Fig. 4) and an association between the presence of and higher lesion burden of pathology and larger entorhinal cortex volumes (Figs. 3 and 5). For *neuritic A β* , we observed an association between higher CERAD A β stage and better performance on the AVLT delayed recall (Fig. 4) and larger hippocampal volumes (Fig. 5). For *vascular pathology*, we observed an association between a higher burden of vascular pathology and larger amygdala and entorhinal cortex volumes (Fig. 5). For *TDP-43*, we observed a higher lesion burden to be associated with larger medial frontal lobe volumes (Fig. 5).

In investigating associations with having at least one pathology as compared with none, we found subjects with at least one

Table 3

Proportion of individuals having each combination of either 2 or 3 pathologies in the entire cohort of 161 clinically normal subjects

Pathology 1	Pathology 2				Pathology 3
	Tau	α -Synuclein	TDP-43	Vascular	
Combinations of any 2 pathologies					
β -Amyloid	40%	12%	14%	28%	NA
Tau		11%	11%	20%	NA
α -Synuclein			3%	5%	NA
TDP-43				8%	NA
Combinations of any 3 pathologies					
β -Amyloid	10%			NA	α -Synuclein
β -Amyloid	8%	2%		NA	TDP-43
β -Amyloid	16%	4%	6%	NA	Vascular
Tau		2%		NA	TDP-43
Tau		3%	4%	NA	Vascular
α -Synuclein			1%	NA	Vascular

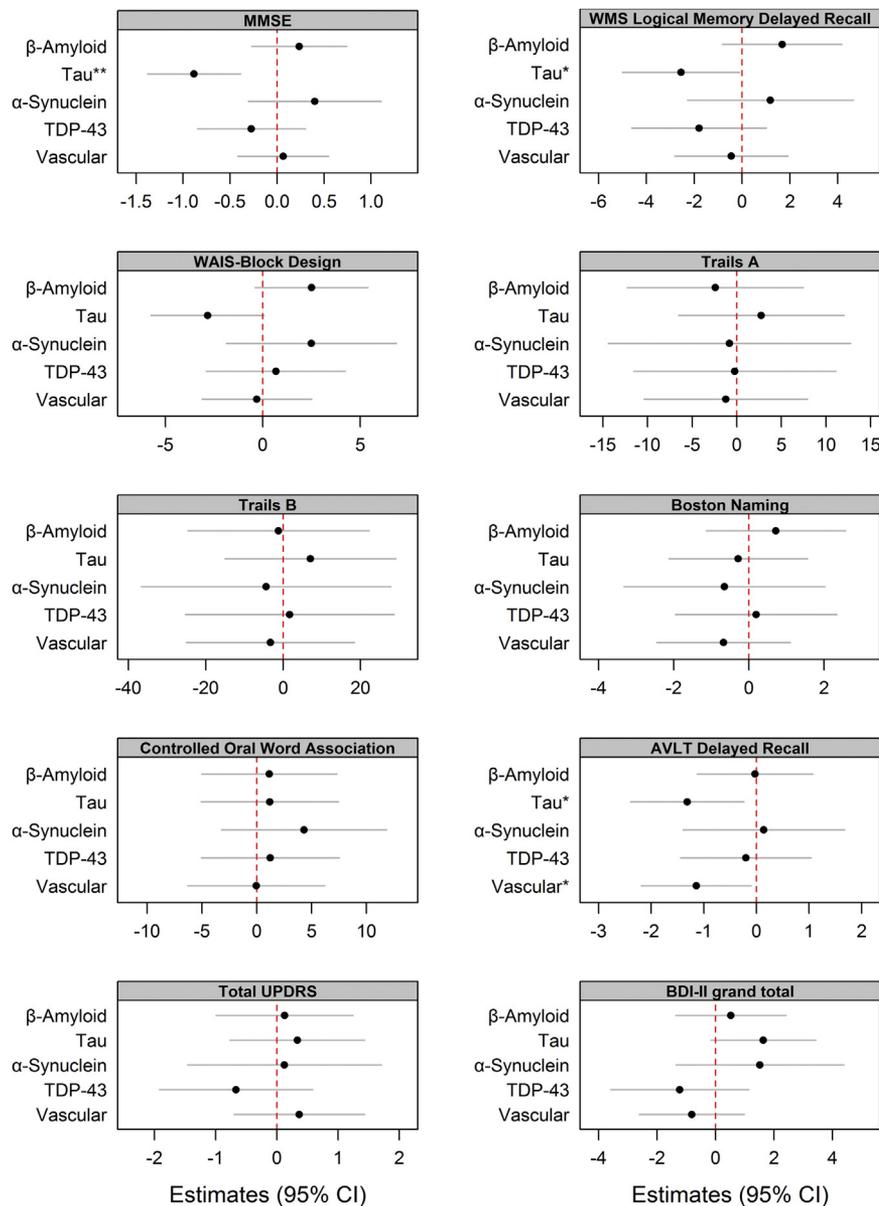


Fig. 2. Plots of estimates from age-adjusted linear regression models with neuropsychological tests as an outcome with pathological measures assessed as binary variables. Models were adjusted for age at neurological examinations. * $p < 0.05$, and ** $p < 0.01$. Abbreviations: AVLT, Auditory Verbal Learning Test; BDI-II, Beck Depression Inventory-II; WMS, Wechsler Memory Scale; UPDRS, Unified Parkinson's Disease Rating Scale; WAIS, Wechsler Adult Intelligent Scale.

pathology were older than subjects with no pathology (Table 4). After adjusting for age, there was an association between the presence of at least one pathology and *APOE* $\epsilon 4$ (Table 4). The odds of having at least one pathology being an *APOE* $\epsilon 4$ carrier was 5.3 [1.3, 39.4] ($p = 0.05$) times higher than the odds of having at least one pathology without being an *APOE* $\epsilon 4$ carrier. There was no association between the presence of having at least one pathology and any clinical measure or brain volume (Table 4). In our trend test assessing the association between a greater number of pathologies and clinical and imaging measures, we found that having a greater number of pathologies was associated with poorer AVLT delayed recall scores [estimate (95% CI) -0.62 ($-1.1, -0.04$)] (Table 5).

4. Discussion

We studied a unique cohort of 161 prospectively followed-up, cognitively and neurologically normal subjects with a CDR-SB

score of 0 at the time of death. We found that the majority had at least one of the dementia-related pathologies, and we found evidence that some of these pathologies influence clinical and anatomic outcomes in these people. We also found associations that could be considered counterintuitive whereby a higher burden/distribution of protein was associated with better clinical performance and/or larger brain volumes.

It has been consistently shown that even if older adults die without a clinical diagnosis of dementia or other impairment, it is likely that they will have some form of dementia-related pathology. In fact, in our cohort, 86% of CN elderly had at least one dementia-related pathology. As observed in our cohort, the most common dementia-related pathologies observed in CN older adults were AD-related pathologies ($A\beta$ and tau) (Rahimi and Kovacs, 2014). The next most common pathology in our cohort was vascular pathology, then TDP-43, and lastly, alpha-synuclein. Similar to AD-related pathologies, vascular pathology is common in CN older adults,

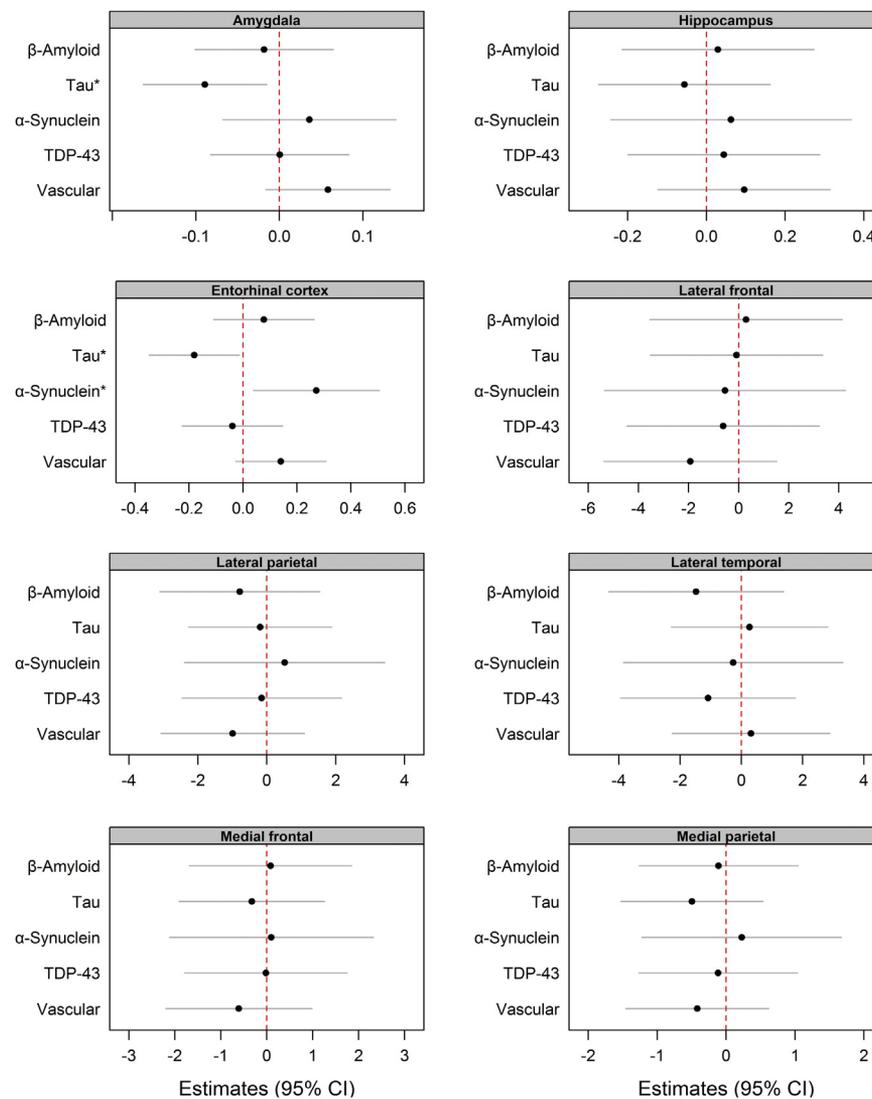


Fig. 3. Plots of estimates from age-adjusted linear regression models with gray matter volumes (cm^3) as an outcome ($N = 102$) with pathological measures assessed as binary variables. Models were adjusted for age at MRI scan, years from MRI scan to death, and log-transformed total intracranial volume. * $p < 0.05$.

but prevalence estimates are broad, with estimates ranging from 33% to 80% in community-based samples (Brayne et al., 2010; Fernando and Ince, 2004; Matthews et al., 2009). TDP-43 prevalence estimates are also wide ranging. A meta-analysis found that TDP-43 was present in 37% of CN older adults in North America (Nascimento et al., 2018). However, among community-dwelling CN older adults, TDP-43 was present in 1% of subjects (Robinson et al., 2018), whereas another study of the oldest old (90 years and older) found TDP-43 to be present in 18% of subjects without dementia (Keage et al., 2014). Hence, the prevalence of TDP-43 seems to be highly dependent on the characteristics of the cohort and is likely also dependent on the regions assessed pathologically for its presence (amygdala vs. hippocampus) (Josephs et al., 2014a). Alpha-synuclein was, indeed, relatively rare in CN older adults in other studies, observed in 5%–12% of cases (Brayne et al., 2010; Knopman et al., 2003; Petrovitch et al., 2005).

Mixed pathologies were very common in our cohort with more than 60% of the subjects having 2 or more pathologies, a third having at least 3 pathologies, and almost 10% having 4 pathologies. It is apparent that some combinations of pathologies are more common than others, with combinations involving A β , tau, and

vascular pathology being the most common. These findings are quite striking given that the subjects in our cohort were cognitively normal with a CDR-SB score of 0 at the time of last evaluation before death. Mixed pathologies have been reported in elderly cohorts consisting of individuals with a range of clinical diagnoses, including dementia (Rahimi and Kovacs, 2014). However, mixed pathologies have not been commonly reported in CN subjects (Bennett et al., 2006; Knopman et al., 2003; SantaCruz et al., 2011; Suemoto et al., 2017), especially when defined as CDR-SB = 0. These findings suggest that cognitive reserve may be playing a role in sparing these individuals from developing clinical symptoms of the disease before death (Stern, 2009). Alternatively, it is also likely that it is the burden of the pathologies that is key as opposed to the number of pathologies when the burdens are low. Although tau and A β were the most common proteins in our cohort, only around 10% had the highest levels of tau burden/distribution (Braak NFT stage V-VI) or A β (+) neuritic plaque burden/distribution (CERAD stage 3), as others have found (Bennett et al., 2006; Knopman et al., 2003). Similar to another study (SantaCruz et al., 2011), we found the majority of those with the highest levels of tau were Braak NFT stage V, with only 2 subjects (1%) being Braak stage VI.

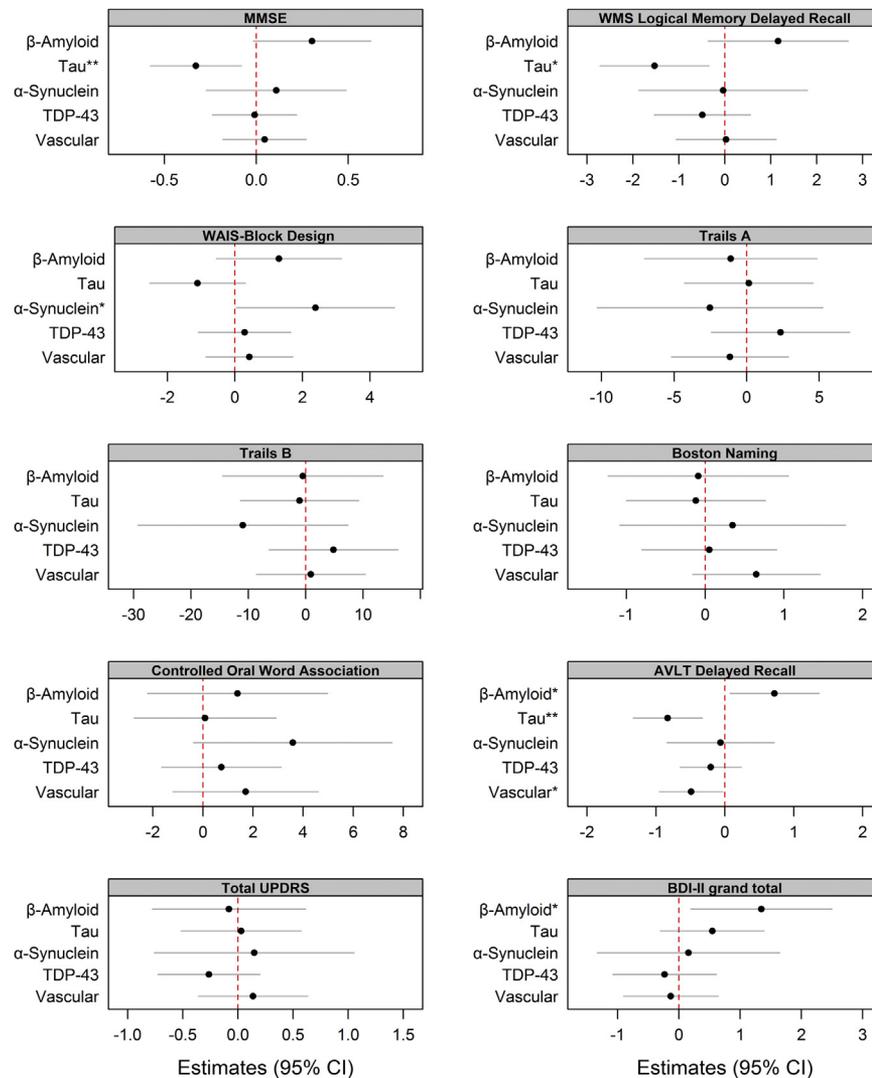


Fig. 4. Plots of estimates from age-adjusted linear regression models with neuropsychological tests as an outcome with pathological measures assessed as ordinal variables. Models were adjusted for age at neurological examinations. * $p < 0.05$, and ** $p < 0.01$. Abbreviations: AVLT, Auditory Verbal Learning Test; BDI-II, Beck Depression Inventory-II; WMS, Wechsler Memory Scale; UPDRS, Unified Parkinson's Disease Rating Scale; WAIS, Wechsler Adult Intelligent Scale.

In our cohort of CN subjects, we found strong associations between tau and clinical measures of episodic memory function, as well as general cognition. These associations were observed when we assessed the presence/absence of tau or tau NFT stage. Hence, the presence of tau and a higher NFT stage were both associated with poorer episodic memory and general cognition. The association between tau and poorer memory is likely related to the association of tau and smaller volumes of medial temporal lobe structures, given that these structures are strongly associated with episodic memory. This is consistent with the fact that these structures are involved in early Braak NFT stages (Braak and Braak, 1991). Another notable finding in our study was the association between the presence, and greater burden, of vascular pathology and lower episodic memory performance. We did not observe any association between vascular pathology and volume of medial temporal lobe structures, suggesting that the influence of vascular pathology on episodic memory is likely independent of medial temporal atrophy. It is possible that the influence from vascular pathology was related to lesions in subcortical gray matter structures, such as the thalamus that has been reported to be associated with episodic memory loss (Aggleton et al., 2016). In the regression analysis when we

treated the variables as ordinal, we observed an association between a higher A β burden of neuritic plaques and higher scores on the BDI-II scale. This was not observed in our binary analysis where we investigated the presence/absence of any A β , that is, neuritic, diffuse, and cored plaques, perhaps suggesting the relationship is specific to neuritic plaque burden. Work in mice has linked A β to depression, with evidence that increased A β production is initiated by a stress response linked to depression (Green et al., 2006).

Although several previous autopsy studies have examined the association of dementia pathologies with clinical measures of cognition in cohorts that include CN individuals, few have investigated this association in a cohort of purely CN individuals (Bennett et al., 2006, 2012; Boyle et al., 2018; Price et al., 2009; SantaCruz et al., 2011; Suemoto et al., 2017). In our cohort, all subjects were clinically diagnosed as being CN and also had a CDR-SB score of 0 at the evaluation closest to death. Hence, our cohort is relatively unique even among studies with CN individuals. One study that assessed the association of A β (+) neuritic and diffuse plaques with cognition in CN subjects identified an association between the presence of neuritic plaques and lower performance in general cognition and memory, after accounting for NFTs (Malek-Ahmadi

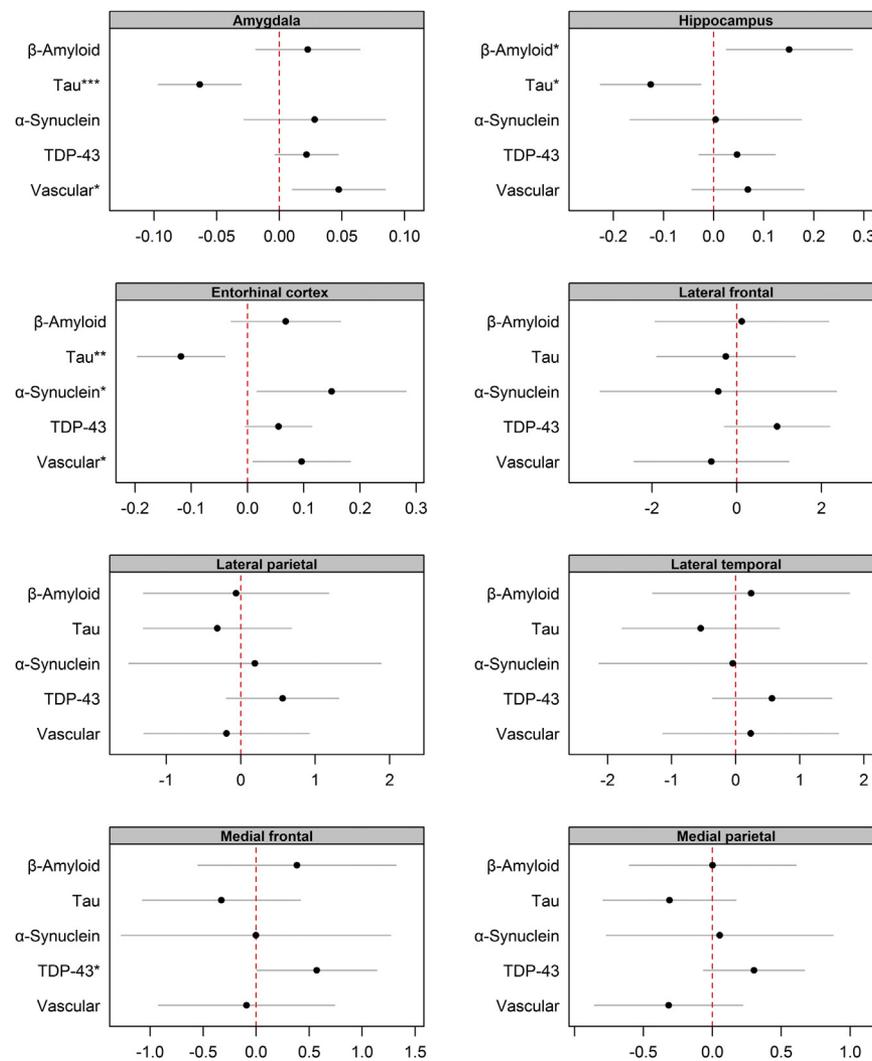


Fig. 5. Plots of estimates from age-adjusted linear regression models with gray matter volumes (cm^3) as an outcome ($N = 102$) with pathological measures assessed as ordinal variables. Models were adjusted for age at MRI scan, years from MRI scan to death, and log-transformed total intracranial volume. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

et al., 2016). Another study that assessed the association of alpha-synuclein pathologies with cognition did not find any association with cognition (Markesbery et al., 2009). Two other studies found an association between higher burden of Alzheimer's pathologies (i.e., A β and/or tau) and lower performance on memory measures (Bennett et al., 2006; Schmitt et al., 2000). One study did find an association between A β and global cognition with only a trend for tangles (Bennett et al., 2012). In our study, however, we identified an association between tau, but not A β , and global cognition similar to another study (Price et al., 2009). The difference in findings could be due to different inclusion criteria or to different methods of analysis. Most autopsy clinicopathologic and imaging pathologic association studies typically combine subjects with and without normal cognition, exclude subjects with dementia but including subjects with varying degrees of mild cognitive impairment, or report on cohorts in which all the subjects were CN at the time of enrollment but of which some later became impaired. For example, a recent longitudinal study that enrolled CN subjects and followed them up to death found associations between clinical trajectories and pathological variables (Nguyen et al., 2018). Yet, although 100% of the cohort was normal at enrollment, 71% were cognitively impaired at death making this cohort very different from ours where 100% of subjects had a CDR-SB score of 0 at death.

Our results demonstrate that tau and vascular pathology were the only pathologies that influenced cognition in CN subjects. Although we did not conduct an analysis in which the relative effect of each pathology was calculated, other groups have attempted this type of analysis with 1 study reporting that tau had the biggest effect on cognition, followed by vascular pathology (Suemoto et al., 2017); however, the cohort in that study included both cognitively impaired and unimpaired subjects and vascular pathology was defined slightly differently from in our study. Another study found that the presence of AD pathology (tau and A β) was the biggest contributor to cognitive decline, followed by vascular pathology, but again in a cohort that included both cognitively impaired and unimpaired subjects (Boyle et al., 2018).

Quite surprisingly, we found many counterintuitive results in our linear regression analyses that used pathological stage, whereby higher stages in all pathologies except for tau were associated with better cognition or larger MRI volumes. Although these findings need to be replicated in a different cohort, it is intriguing to postulate that this may support the theory of cognitive or brain reserve (Stern, 2009). Specifically, our findings may support the notion that having cognitive reserve or starting off with larger brain volumes helps to protect the individual from dropping below the threshold in which signs and symptoms develop in the

Table 4
Comparisons between subjects without pathology and subjects who had at least one type of pathology

	No pathology (N = 23)	At least one pathology (N = 138)
Female, N (%)	13 (57%)	73 (53%)
Age at clinical examination, y	84 (77, 86)	87 (83, 90)**
Age at MRI, y	82 (77, 87)	87 (82, 90)
Age at death, y	84 (78, 89)	90 (85, 94)**
MRI to death, y	4.0 (1.3, 5.9)	3.0 (1.4, 5.6)
Postmortem interval, h	14 (8, 20)	15 (10, 20)
Clinical examination to death, yrs	1.0 (0.5, 1.6)	1.6 (0.7, 3.5)*
Education, y	14 (12, 16)	14 (12, 16)
APOE ϵ 4 carrier, N (%)	2 (9%)	35 (25%)*
23	3 (13%)	19 (14%)
24	1 (4%)	2 (1%)
33	18 (78%)	84 (61%)
34	1 (4%)	29 (21%)
44	0 (0%)	4 (3%)
Clinical scores	No pathology (N = 23)	At least one pathology (N = 138)
Mini-Mental State Examination score/30	29 (27, 29)	28 (27, 29)
WMS Logical Memory Delayed Recall/50	21 (16, 25)	17 (12, 23)
Boston Naming/60	56 (54, 58)	54 (51, 57)
Controlled Oral Word Association Test	34 (25, 43)	36 (29, 45)
WAIS-Block Design/51	20 (16, 27)	20 (14, 25)
AVLT Delayed Recall/15	8 (4, 11)	6 (4, 8)
Trails A/180	47 (36, 56)	46 (36, 64)
Trails B/300	114 (83, 140)	126 (95, 170)
Total UPDRS/44	1 (0, 1)	1 (0, 2)
BDI-II/63	4 (2, 6)	6 (2, 9)
Gray matter volumes (cm ³)	No pathology (N = 12)	At least one pathology (N = 90)
TIV (l)	1.57 (1.46, 1.60)	1.49 (1.39, 1.60)
Amygdala	2.07 (1.93, 2.11)	1.96 (1.78, 2.17)
Hippocampus	7.35 (6.70, 7.48)	6.97 (6.42, 7.52)
Entorhinal cortex	3.03 (2.77, 3.37)	2.82 (2.44, 3.30)
Lateral frontal	55 (51, 71)	55 (50, 60)
Lateral parietal	37 (32, 43)	34 (31, 38)
Lateral temporal	68 (63, 77)	64 (60, 70)
Medial frontal	29 (26, 33)	27 (25, 30)
Medial parietal	20 (17, 22)	18 (17, 21)

Unless indicated otherwise, data shown are median (IQR).

* = $p < 0.05$, ** = $p < 0.01$.

Key: APOE, apolipoprotein E; AVLT, Auditory Verbal Learning Test; BDI-II, Beck Depression Inventory-II; WMS, Wechsler Memory Scale; TIV, total intracranial volume; UPDRS, Unified Parkinson's Disease Rating Scale (modified version used); WAIS, Wechsler Adult Intelligent Scale.

presence of high neuropathological stages. Alternatively, the findings could be driven by the fact that we restricted our cohort to CN subjects and excluded subjects with cognitive impairment. For example, considering the counterintuitive relationship between A β and hippocampal volume and given that greater Braak NFT stage was related to smaller hippocampal volumes; if a subject has high NFT stage and high A β stage, then it may be that the only way they could remain CN is if they have preserved hippocampal volume because the presence of hippocampal atrophy in addition to high tau and A β stage would likely render them cognitively impaired. We refer to this as the “triangular effect.” Following the same thinking, the results could also be telling us that a relationship between tau and hippocampal volume alone is not sufficient to render a subject cognitively impaired in the absence of a relationship between other pathologies and medial temporal volumes. Supportive of this hypothesis was the fact that the trend test showed that performance on the AVLT was related to the number of pathologies present in the brain. In addition to the tau relationships, one of the other pathologies may need to exert an influence for a subject to become

Table 5
Trend test of a number of pathologies

	Trend test	
	Est. (95% CI)	p-values
Clinical scores		
MMSE/30	-0.12 (-0.34, -0.01)	0.29
WMS Logical Memory Delayed Recall/50	-0.30 (-1.4, -0.10)	0.53
Boston Naming/60	-0.10 (-0.89, -0.15)	0.81
Controlled Oral Word Association	0.65 (-2.4, 0.72)	0.67
WAIS-Block Design/51	0.22 (-1.0, -0.23)	0.73
AVLT Delayed Recall/15	-0.62 (-1.1, -0.04)	0.01
Trails A/180	-0.30 (-4.2, 2.1)	0.88
Trails B/300	0.38 (-8.9, 5.2)	0.94
Total UPDRS/44	0.24 (-0.29, 0.17)	0.37
BDI-II/63	0.60 (-0.18, 0.20)	0.13
Gray matter volumes (cm ³)		
Amygdala	-0.007 (-0.04, 0.03)	0.68
Hippocampus	0.03 (-0.06, 0.10)	0.50
Entorhinal cortex	0.04 (-0.03, 0.10)	0.26
Lateral frontal	-0.60 (-2.0, 0.90)	0.43
Lateral parietal	-0.40 (-1.0, 0.40)	0.32
Lateral temporal	-0.40 (-1.0, 0.70)	0.47
Medial frontal	-0.20 (-0.90, 0.50)	0.55
Medial parietal	-0.20 (-0.70, 0.20)	0.29

Significant p-value is shown in bold.

Key: APOE, apolipoprotein E; AVLT, Auditory Verbal Learning Test; BDI-II, Beck Depression Inventory-II; WMS, Wechsler Memory Scale; TIV, total intracranial volume; UPDRS, Unified Parkinson's Disease Rating Scale (modified version used); WAIS, Wechsler Adult Intelligent Scale.

cognitively impaired, that is, the effects of multiple pathologies are necessary. However, it is important to remember that the neuropathological stages themselves are not perfect surrogates of pathological burden. The fact that we did not see these counterintuitive associations when we analyzed the presence/absence of pathologies may tell us that the binary analysis lacks sensitivity.

We found that APOE ϵ 4 carriers were more likely to have at least one type of pathology than noncarriers. This may be driven mainly by the well-known association between APOE ϵ 4 and A β , particularly in younger individuals. With that said, we cannot exclude the possibility that APOE ϵ 4 is also playing a role in the deposition of the other 4 pathologies. Indeed, we and others recently reported an association between the APOE ϵ 4 allele and Lewy body disease (Dickson et al., 2018) and between APOE4 and TDP-43 (Wennberg et al., 2018; Yang et al., 2018), independent of A β pathology.

There are limitations of the study that should be mentioned. Although the CDR-SB score was determined to be 0 at the time of the last examination before death, it is possible that it could have changed between that time and death, which was approximately 1.5 years. Another limitation is the fact that we did not consider the location of the vascular infarcts, which may have provided more granularity in the results.

Although past imaging studies have shown that CN aged individuals have dementia pathology, including some of the pathologies examined in this study and vascular pathology, it is important to consider that because these studies are conducted in vivo, it is not known whether or not the individuals will develop dementia before death. Our study indicates that even if an individual dies CN, dementia-related protein deposition and vascular pathologies are associated with poorer memory performance and depression. Moreover, we found that the APOE ϵ 4 allele appears to be a risk factor for having at least one of the 5 types of pathology measured here. Furthermore, although imaging technology is quite advanced, there is still debate as to whether we are able to precisely measure the correct forms of A β and tau in vivo and how much off-target binding contributes noise (Klunk, 2018). In addition, we are not currently able to measure alpha-synuclein and TDP-43 using imaging. Therefore, histological studies at autopsy are very important and may provide us with a more accurate measure of these proteins.

Disclosure

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Authors' contributions: AMW was responsible for data interpretation and drafting of the original report. KAJ and JLW were responsible for the concept and design of the study, data interpretation, and manuscript revision, which was also reviewed and revised by all coauthors. KAJ, MEM, and DWD were responsible for TDP-43 acquisition data. NT and SDW were responsible for statistical analysis and all figures and tables. LP was responsible for providing the TDP-43 antibodies used in this study. RCP and DSK were responsible for recruiting the cohort, clinical evaluations, and APOE genotyping. Pathological examinations were done by DWD and JEP. Funding was obtained by KAJ and RCP. All authors contributed to the editing and writing of the manuscript.

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